Screening of Parental Lines of Three- Line Rice Hybrid against *Xanthomonas Oryzae* pv. *oryzae*

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Abstract Experiments were conducted in pot in two seasons to screen some parental lines of hybrid rice against *X. oryzae pv. oryzae*. Fifty parental lines (Maintainer and Restorer lines) and 2 check varieties viz. 'Purbachi' and 'TN 1' as susceptible check were used as test materials. BXO9, the most virulent isolate of *X. oryzae* pv. *oryzae* in Bangladesh was used for inoculation. The inoculation was done by leaf clipping method. Based on lesion length on leaves and relative lesion length the parental lines were grouped into five clusters. In aman season (July to December) 14 days after inoculation, among the five clusters, cluster I contained 21 lines including 'Purbachi' and 'TN 1'. Cluster III comprised 4 lines which contain resistant parental line 'BR 168-2B-283R'. In boro season (November to May), on the other hand, parental line 'IR 68885' showed resistant reaction which was placed in cluster I comprising a total of 10 lines. But 'Purbachi' and 'TN 1' were grouped in cluster IV containing 5 lines. Therefore, it was found that the line 'BR 168-2B-283R' and 'IR 68885' showed resistant reaction during aman (July to December) and boro (November to May) seasons respectively.

Keywords: Bacterial leaf blight, CVA, Hybrid rice, Inoculation, Lesion length, parental lines.

Introduction

Rice (*Oryzae sativa* L.), belonging to the family Poaceae is widely cultivated in most tropical and subtropical regions of the world (Ezuka and Kaku 2000). Rice is one of the major food crops of the world especially of the Asian countries like Bangladesh, Pakistan, India China, Vietnam and Korea. More than 90% of the world's rice is produced and consumed in Asia (Virmani 1996) and constitutes a staple food for 2.7 billion people worldwide (Salim *et al.*, 2003). Bangladesh ranks fourth with respect to production and consumption of rice in the world, with annual production 123.13 lac metric ton (BBS, 2006;

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Krishi dairy, 2006). Rice provides 75% of the calories and 55% of the protein in the average daily diet of the people (Bhuiyan et al., 2002). Compared to mid sixties rice production in Bangladesh has almost doubled during the last three decades. It is however unfortunate that such an important crop is attacked by many kinds of diseases. Generally, rice crop is threatened by more than 40 diseases and that is one of the reasons for low yield of rice in the world including Bangladesh. Certain diseases and pests are more prevalent on hybrid rice than on conventional varieties of which bacterial leaf blight (BLB) caused by Xanthomonas oryzae is one of the most destructive diseases throughout the world (Mew, 1987). This disease was first noticed by the farmers in Fukuoka prefecture Kyushu Island, Japan, as early as in 1884-85 (Ezuka and Kaku, 2000). The pathogen normally enters the host through wounds or natural openings such as the water pores on hydathodes (Mew, 1987) and multiplies in the tissues of the epitheme, into which xylem vessels open (Tabei, 1997). After the bacterial multiplication occurs in epitheme, some of the bacterial cells reach the xylem vessels of the vascular system (Li et al., 2001). When a bacterial colony has established in the xylem vessels, the bacterial cells grow vigorously and translocate through the network of the vascular system (Ezuka and Kaku, 2000).

Bacterial blight is also a serious problem of hybrid rice production because of the susceptibility of the parental lines to the pathogen populations under field conditions (Shahjehan *et al.*, 1991). In some areas of Asia, it can reduce crop yield up to 50% (Khush and Ogawa, 1989) and even up to 80% (Singh *et al.*, 1977). The disease may weaken the seedling and in older plants the loss of grain may be 4.578-29.1% (Bedi and Gill, 1960). including Bacterial leaf Blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama, 1922). Bacterial leaf blight of rice is one of the most destructive diseases of rice throughout the world (Swings *et al.*, 1990). BLB is the most serious disease in South Asia (Ou 1985) and in many Asian countries bacterial leaf blight has become endemic on rice following repeated cultivation (Mew *et al.*, 1993).

Host Plant resistance is an important component of an integrated management program for this disease. To minimize the risk of attack by bacterial blight, evolving resistant cultivars against the pathogen is the best non chemical method for management of the disease. To develop high yielding varieties with durable resistance to bacterial blight, it is necessary to understand the population structure of this pathogen. The existing population of the bacterium was classified into virulence groups/races based on their interaction with differential cultivars by various workers (Das *et al.*, 1994; Ou, 1985).

Although many sources of resistance to bacterial leaf blight have been identified in rice growing countries in Asia (Khush and Ogawa, 1989; Ogawa *et al.*, 1991) resistant breeding to *X. oryzae* pv. *oryzae* in Bangladesh is still in an early developmental stage (Khan *et al.*, 2009). Breeding for durable resistance to bacterial leaf blight requires recent information on the pathogen population and resistant source(s) of the parental line of rice against bacterial leaf blight pathogen for hybrid rice production in Bangladesh. Therefore, the present study was undertaken to identify resistant parental lines against bacterial leaf blight (BLB) which may be used for developing new hybrid rice varieties.

Materials and methods

The experiments were conducted in the Plant Pathology Field Laboratory and at the Seed Pathology Center, Bangladesh Agricultural University, Mymensingh-2202 during during two consecutive rice cultivation seasons prevailing in Bangladesh viz. aman season and boro season from 26 July, 2011 to 22 May, 2012.

Collection of test materials and seed sowing

Fifty parental lines of rice (B and R lines) was obtained from the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh and two check rice varieties ('Purbachi' and TN 1 as susceptible check) from the Plant Pathology Division, BRRI, Joydebpur, Gazipur. Seeds were sown in the nursery bed maintaining line to line distance 10 cm and line length 1 m with continuous seeding in line. Susceptible check (Purbachi and TN 1) was seeded at every 10 lines interval. At 60 days after seeding, seedlings were used for transplanting for pot experiment. Seedlings were planted in 52 pots maintaining 4 hills of each entry in a pot and susceptible checks (Purbachi and TN 1). When the growth of the plant reached in around maximum tillering stage, all the test lines were inoculated with BXO9 (Bacterial blight isolate).

Bacteria strain revival

Bacteria were grown on single media for revival i.e. Peptone Sucrose Agar (PSA) medium. The pouring and inoculation were carried out under laminar air flow cabinet. These plates were incubated at 28°C for two to three days depending upon colony development. The composition of PSA medium was: peptone: 10g, sucrose: 10g, agar: 20g and water: 11iter.

Preparation of bacterial suspension and Inoculation of parental materials

For the preparation of fresh inoculum, 10 ml of sterile water was added to the 48 hours culture of *X. oryzae* pv. *oryzae* in the slants for the preparation of bacterial suspension. The leaf clipping method as described by Kauffman *et al.* (1973) was followed in this experiment for inoculation. In this method, a pair of sterilized surgical scissors was dipped in bacterial suspension. Leaves of all the plants in a pot were grasped in one hand and the top 1-3 inches of leaves were clipped off simultaneously with the scissors. The same procedure was followed for inoculation of each parental line of rice.

Collection and Analysis of data

A total of 20 inoculated rice leaves were randomly selected from each entry in pot experiment. Data on lesion length caused by Bacterial blight pathogen on inoculated leaves and relative lesion length were recorded at 14 days after inoculation (DAI). Lesion length was measured by measuring scale. The relative lesion length was computed using the following formula as described by Fang *et al.* (1990):

Relative lesion length $\% = \frac{\text{Lesion length}}{\text{Length of the leaf}} \times 100$

Mean data of each character was subjected to multivariate analysis following Principal component analysis (PCA), Canonical vector analysis (CVA) and Cluster analysis (CLSA) using GENSTAT 5.

Results and discussion

Reaction of parental lines to the BLB pathogen during aman season (July to December)

Mean values of lesion length and relative lesion length by *X.oryzae pv.oryzae* during aman season at 14 days after inoculatio (DAI) are shown in Table.1. Variations in two parameters related to BLB on 52 genotypes indicated the existence of genetic diversity among the tested parental materials. The lesion length of BLB ranged from 1.61 to 27.56 cm among the genotypes. The lowest lesion length was found on line 'BR 168-2B-283R' (1.61 cm) and that of the highest on 'IR 60819-34-2-1R' (27.56 cm) followed by the susceptible

check 'Purbachi' (25.94 cm.). As regard to relative lesion the highest was recorded from the line 'TJ-10B' (98.08%) followed by 'Purbachi' (97.89 %.) and the lowest value of the parameter was found on 'BR 168-2B-283R' (7.32 %). However the range for this papameter varied from 98.08 to 7.32% among the lines.

The parental line showing lower lesion length of less than 3.0 cm was considered as resistant to BLB. In case of race specific reactions of 52 materials tested, 'BR 168-2B-283R' showed resistant reaction to the most virulent race of *X. oryzae* pv. *oryzae* tested in the present experiment. In addition 'V20 B', 'BRRI 10 B' also showed resistant reaction to the disease (Table.1).

Reaction of parental lines to the BLB pathogen during boro season (November to May)

Mean values of lesion length and relative lesion length by *X. oryzae* days are shown in Table 1. The lesion length of BLB on the genotypes ranged from 0 to 12.00 cm. The lowest lesion length was found on 'IR 68885' (no symptom) and the highest lesion length was found on susceptible check 'TN 1' (12.00 cm) followed by the susceptible check 'Purbachi' (10.83 cm.). The relative lesion length ranged from 0 to 37.50%. The highest relative lesion length was recorded from 'TN 1' (37.50%) followed by 'Purbachi' (32.17 %.). The lowest value of the parameter was found on 'IR 68885'.

The parental lines showing lower lesion length of less than 3.0 cm was considered as resistant to BLB. In case of race specific reactions of 52 materials tested, 'IR 68885' showed resistant reaction to the most virulent race of *X. oryzae* pv. *oryzae* tested in the present experiment. In addition 'IR74653-115-1-2-1', 'BAU-6B', '85 B₂', 'IR 75304--39-1-2-1', 'IR64608B', 'BRRI 10B-2', 'IR 68888B, 'IR 67684 B', 'IR 69622B', 'Jin B', 'IR 68899B', 'IR 62829 B', 'ZS 97 B', 'TJ-13B', 'IR69626B', 'V20 B', 'BRRI 10 B', 'BRRI 1B', 'BRRI 10B-2(2)', 'IR 13155-60-3-1-3R', 'IR 29729-143-3-2-1R', 'IR 58082-126-1-2R', 'IR 58110-144-2-2-2R', 'IR 62171-122-3-2-3-2R', 'IR 63870-123-2-2-2R', 'IR 65489 H-AC2-2R', 'BR 736-20-3-1R', 'BR 168-2B-283R', 'IR 13149-43-2-P-1R', 'IR 9761-19-1R', 'IR 65515-56-1-3-19R', 'IR 24' also showed resistant reaction to the disease (Table.1).

Genetic diversity among the tested parental lines

Non-hierarchical clustering of genotypes

The computations from distance matrix gave non-hierarchical clustering of 50 parental lines including 2 check varieties and grouped them into five different clusters (Table 2 and Table 3) based on the data obtained during aman and boro season.

Analysis of data obtained in aman season gave five clusters of which cluster I comprised the highest number of 21 parental lines followed by cluster II and cluster IV. Cluster I, II, III, IV and cluster V consisted of twenty one, nine, four, eleven and seven lines respectively. Among the tested materials, resistant parental line 'BR 168-2B-283R' was placed in cluster III. The inclusion of susceptible check 'TN 1' and 'Purbachi' in cluster I indicated that these genotypes is totally different from other lines used in this study (Table 2).

On the other hand, the distribution pattern based on boro season data indicated that cluster V comprised of the highest number of 16 tested lines followed by cluster II and cluster I. Cluster I, II, III, IV and cluster V comprised of ten, thirteen, eight, five and sixteen lines respectively. Among the tested materials, resistant parental line 'IR 68885' was placed in cluster I. The inclusion of susceptible check 'TN 1' and 'purbachi' in cluster IV indicated that this variety is totally different from other lines used in this study (Table 3).

Canonical variant analysis (CVA) of the data obtained in aman season (July to December)

Canonical Variant Analysis (CVA) was done to compute the intra- (bold) and inter-cluster distances (D^2) values. Statistical distances represented the index of genetic diversity among the clusters.

The inter cluster distance was maximum between cluster I and cluster III (17.500) followed by the distance between cluster I and cluster V (11.794). The minimum inter cluster distance was observed between cluster I and cluster II (4.012). The highest intra-cluster distance was noticed from the cluster III (0.819) followed by cluster V (0.409) and the lowest being in cluster II (0.237). However, differences in cluster means existed for almost all the characters studied. In cluster III, it contained lowest value of characters considered viz. lesion length and relative lesion length and conversely, cluster I contained the highest value of those parameter (Table. 4).

The intra-cluster distances ranged from 0.237 to 0.819 (Table. 4). Intracluster distances in all clusters were more or less low which indicated genotypes within the same cluster were closely related. The highest intracluster distance was recorded in cluster III containing four genotypes followed by cluster V. The lowest intra-cluster distance was observed in cluster II having 9 parental lines. It was revealed that intra-cluster diversity was the highest in cluster III *i.e.*, more heterogeneous and intra-cluster diversity was the lowest in cluster II *i.e.* comparatively homogenous. Higher inter and intra cluster distances indicate higher genetic variability among genotypes between and within clusters, respectively. The minimum inter and intra cluster distance indicates closeness among the genotypes of two clusters and within a cluster.

Cluster mean value of 2 different characters viz. mean lesion length and relative lesion length are shown in Table. 5. The highest mean lesion length and relative lesion length was found in cluster I and lowest in cluster III.

Canonical variant analysis (CVA) of the data obtained in boro season (November to May)

Canonical Variant Analysis (CVA) was done to compute the intra- (bold) and inter-cluster distances (D^2) values.

The inter cluster distance was maximum between cluster I and cluster IV (16.786) followed by the distance between cluster I and cluster V (7.735). The minimum inter cluster distance was observed between cluster I and cluster III (1.725). The maximum intra-cluster distance was noticed from the cluster I (0.528) followed by cluster V (0.340). The minimum was found in cluster II (0.214) followed by cluster III (0.215). Differences in cluster means existed for almost all the characters studied. In cluster I, it contained lowest value of all the studied characters (lesion length and relative lesion length) and cluster IV contained the highest value (Table. 7).

The intra-cluster distances ranged from 0.214 to 0.528 (Table.7). Intracluster distances in all clusters were more or less low which indicated genotypes within the same cluster were closely related. The highest intracluster distance was recorded in cluster I containing ten genotypes followed by cluster V. The lowest intra-cluster distance was observed in cluster II having thirteen parental lines. It was favored to decide that intra-cluster diversity was the highest in cluster I *i.e.*, more heterogeneous and intra-cluster diversity was the lowest in cluster II *i.e.* comparatively homogenous.

Higher inter and intra cluster distances indicate higher genetic variability among genotypes between and within clusters, respectively. The minimum inter and intra cluster distance indicates closeness among the genotypes of two clusters and within a cluster.

Cluster mean value of 2 different characters viz. mean lesion length and relative lesion length are shown in Table.8. The highest mean lesion length and relative lesion length was found in cluster IV and lowest in cluster I.

Contribution of different characters towards divergence of the genotypes

Vector I and Vector II values were obtained from Principal Component Analysis (PCA). In first axis vector I, mean lesion length had negative impact; and relative lesion length had positive impact towards divergence. In vector II, mean lesion length had positive impact; and relative lesion length had negative impact towards divergence (Table.6).

With regard to the boro season experiment, relative contribution towards divergence is presented in Table. 9. Vector I and Vector II values were obtained from Principal Component Analysis (PCA). In both axis vector I and vector II, mean lesion length had negative impact; and relative lesion length had positive impact towards divergence.

Construction of scatter diagram

On the basis of principal axes I and II from the principal component analysis, a two dimensional scatter diagram using component score I as X-axis and component score 2 as Y-axis was constructed (Fig. 1). The distribution of genotypes in scattered diagram was distributed into 5 clusters revealing the existence of considerable diversity among the parental lines.

Variation in mean Bacterial Leaf Blight severities of the tested 52 materials (50 parental lines and 2 check varieties i.e. TN 1 and Purbachi as susceptible check) to the most virulent race (BXO9) of BLB pathogen (*X. oryzae* pv. *oryzae*) were found. It indicated the existence of the genetic variability of BLB resistance among the tested materials.

Among the tested materials, resistant parental line 'BR 168-2B-283R' was placed in cluster III along with V 20B, BRRI 10B and IR 63870-3-2-3-3R. The inclusion of susceptible check 'Purbachi' and 'TN 1' in cluster I indicated that this parental line is totally different from other lines used in this study (Table 8). Due to the lowest intra cluster means for mean lesion length and relative lesion length are obtained from cluster III (Table 4). Therefore, more emphasis should be given on this cluster for selecting BLB resistant rice parental lines, which may be useful for the development of hybrid BLB resistant rice cultivars.

In the present study, relative lesion length showed positive value in vector I and mean lesion length showed positive value in vector II and thus, it contributed most towards divergence. Mean lesion length in vector I and relative lesion length in vector II showed negative value indicating the character contributed lowest for divergence in the studied materials.

Among the 52 materials tested against BLB pathogen at maximum tillering stage, BR 168-2B-283R and BRRI 10B may select for using resistant sources because of their lowest response to the BLB pathogen which belong to cluster III.

But, the highest intra-cluster distance was recorded in cluster III followed by cluster V. It was favored to decide that intra-cluster diversity was the highest in these clusters *i.e.*, materials of these clusters were more heterogeneous. Selection of materials from the heterogeneous cluster may not beneficial for crossing programme.

Genotypes belonging to the distant clusters could be used in hybridization program for obtaining a wide spectrum of variation among the segregates (Mokate *et al.*, 1998). It is more beneficial if crossing might be carried out between genotypes belonging to different groups if their genetic distances (D^2) are greater than 12.5 (Wei *et al.*, 1994).

In the present study, the inter cluster distances between cluster I and IV with other cluster suggesting that crossing of genotypes of cluster I and IV with desirable genotypes of other clusters would express heterotic effect.

Other researchers from Nepal (Karki, 1991), China (Zhang *et al.*, 1994; Zhao *et al.*, 1994), India (Mohanty *et al.*, 1996), Bangladesh (Mondal and Hossain, 1997; Khan *et al.*, 2009), and Pakistan (Ali *et al.*, 2009; Shah *et al.*, 2009) screened large number of genotypes and found rice genotypes with resistant genes against the Bacterial Leaf Blight pathogens.

Breeding for durable resistance to bacterial leaf blight requires recent information on the pathogen population and resistant source(s) of the parental line of rice against BLB pathogen for hybrid rice production in Bangladesh. The findings of the present study clearly showed that, several resistant source(s) present in parental lines. Except these tested materials, other parental lines remain to be tested for further confirmation. Therefore, it is necessary to screen more parental lines which show resistance both in aman and boro season against *Xanthomonas oryzae* pv. *oryzae* for the selection of resistant source(s) for hybrid rice production. So, further research is necessary for the confirmation of these materials as resistant sources both in aman and boro season using Marker Assisted Selection (MAS) using specific gene based markers.

Sl. No. 1	Name of parental lines	Aman S	Season	Bor	o Season
	· ·	Mean lesion	Relative lesion	Mean lesion	Relative lesion
		length (cm)	length (%)	length (cm)	length (%)
01 I	Deshan B	15.11	84.89	8.67	29.63
02 I	IR68885	15.38	93.78	0	0
03	You-1B	12.94	91.13	3.00	12.16
04 I	IR72083B	17.15	89.79	4.00	14.29
05 I	IR74653-115-1-2-1	18.83	98.08	2.67	9.42
06 I	BAU-6 B	16.11	65.99	1.83	7.04
07 I	IR68885	13.44	86.65	1.67	6.55
08 1	IR75304-39-1-2-1	10.77	69.04	0.58	1.78
09 I	IR64608B	12.56	75.53	0.83	2.66
10 I	BRRI 10B-2	11.17	61.37	1.50	4.64
11 I	IR 68888B	8.89	63.5	1.50	4.12
12 I	IR 67684 B	12.15	72.32	4.00	12.9
13 I	BRRI 10B-1	13.28	86.23	1.75	6.36
14 I	IR 69622B	8.61	55.19	10.00	31.25
15 J	Jin B	15.67	89.03	2.17	8.8
16 I	IR 68899B	12.67	66.68	1.00	4.51
17 I	IR 62829 B	11.39	71.19	1.67	5.06
18 5	ZS 97B	13.83	70.56	2.00	7.14
19 I	IR 72909-87-3-2-3	10.78	67.38	0.90	2.39
20	IR 58025B	13.67	94.28	4.67	16.48
21	IR69626B	13.39	86.39	1.83	7.04
22	V 20B	2.89	11.89	0.33	1.02
23 1	BRRI 10B	2.78	8 35	0.55	1.63
24	BRRI 1B	3.72	35.28	0.33	1.39
25 1	BRRI 10B-2 (2)	811	37.00	2.16	7.28
2.6	Ajava R	10.00	52.36	3.00	13.53
27	IR 13155-60-3-1-3R	5.61	34.84	0.83	2.86
28	IR 29729-143-3-2-1R	12.00	61.22	1.50	4 64
29	IR 58082-126-1-2R	8 78	46.95	2.50	7.50
30	IR 58110-144-2-2-2R	5.83	30.05	1.50	5.92
31	IR 59624-34-2-2 R	17.44	63.42	4.33	15.59
32	IR 59682-132-1-1-2R	21.22	91 47	3.83	12.09
33 5	SN 10A	23.22	89.30	7.83	29.73
34	IR 62171-122-3-2-3-2R	17.28	90.95	2.50	12.3
35 1	IR 63870-123-2-2-2-2R	16.33	83 32	2.50	9.89
36	IR 63870-3-2-3-3R	6 44	23.68	4 00	13.48
37	IR 65489 H-AC2-2R	20.17	91.68	2.33	7.68
38 1	IR 68926-61-1R	17.78	77 30	6.00	20.46
39	BR 736-20-3-1R	8.28	39.24	0.67	2.36
40 1	IR 44675 R	17.22	64.25	4 00	14 72
41	BR 168-2B-283R (*)	1.61	7.32	0.25	0.77
42 1	IR 65515-19R	20.28	90.82	3.67	13.76
43 1	IR 13149-43-2-P-1R	17 78	65.13	1 33	4 22
44	IR 9761-19-1R	19.17	70.35	2 67	8.09
45 1	IR 60819-34-2-1R (**)	27.56	86.13	7.83	22.37
46 1	IR 60236-222-3-3-1-2R	16.61	86.83	3.67	13.85
47 I	IR 63879-195-2-2-3-2R	16.44	72 74	3.00	11 84
48 1	IR 65515-56-1-3-19R	10.17	41 51	0.83	3 83
49 I	IR 74	14 94	72.88	1.83	6.24
50 1	IR 36	21.56	92.93	6.00	17 14
51 1	Purbachi	25.94	97.89	10.83	32 17
52	TN 1	22.77	94.88	12.00	37.5

Table 1. Disease development on 50 parental lines and 2 check rice varieties at 14 DAI by *X. oryzae* pv. *oryzae* during aman season

Note: (*) indicates lowest lesion length and (**) highest lesion length among the tested materials.

Table 2. Distribution of 50 Parental lines with 2 check varieties in five cluster (during aman season 14 DAI)

Cluster	Member	Name of variety
Ι	21	Deshan B, IR 68885, You-1B, IR72083B, IR74653-115-1-2-1, 85 B ₂ ,
		BRRI 10B-1, Jin B, IR 58025B, IR69626B, IR 59682-132-1-1-
		2R, SN 10 A, IR 62171-122-3-2-3-2R, IR 63870-123-2-2-2R,
		IR 65489 H-AC2-2R , IR 65515-19R , IR 60819-34-2-1R, IR
		60236-222-3-3-1-2R, IR 36, Purbachi, TN 1
II	9	IR 7530439-1-2-1, IR64608B, IR 67684 B, IR 62829 B, ZS 97B, IR
		68926-61-1R, IR 9761-19-1R, IR 63879-195-2-2-3-2R, IR 24
III	4	V 20B, BRRI 10B, IR 63870-3-2-3-3R, BR 168-2B-283R
IV	11	BAU-6B, BRRI 10B-2, IR 68888B, IR 69622B, IR 68899B, IR
		72909-87-3-2-3, Ajaya R, IR 29729-143-3-2-1R, IR 59624-34-2-2 R,
		IR 44675 R, IR 13149-43-2-P-1R
V	7	BRRI 1B, BRRI 10B-2 (2), IR 13155-60-3-1-3R, IR 58082-126-1-2R,
		IR 58110-144-2-2-2R, BR 736-20-3-1R, IR 65515-56-1-3-19R

Table 3. Distribution of 50 Parental lines with 2 check varieties in five cluster (during boro season 14 DAI)

Cluster	Member	Name of variety
Ι	10	IR 68885, IR 75304-39-1-2-1, IR64608B, IR 72909-87-3-2-3,
		V 20 B, BRRI 10B, BRRI 1B, IR 13155-60-3-1-3R, BR 736-
		20-3-1R, BR 168-2B-283R
II	13	IR74653-115-1-2-1, BAU-6B, 85 B ₂ , BRRI 10B-1, Jin B, ZS 97
		B, IR69626B , BRRI 10B-2 (2), IR 58082-126-1-2R , IR
		63870-123-2-2-2R, IR 65489 H-AC2-2R, IR 9761-19-1R,
		IR 24
III	8	BRRI 10B-2, IR 68888B, IR 68899B, IR 62829 B, IR 29729-
		143-3-2-1R, IR 58110-144-2-2-2R, IR 13149-43-2-P-1R,
		IR 65515-56-1-3-19R
IV	5	Deshan B, IR 69622B, SN 10 A, Purbachi, TN 1
V	16	You-1B, IR72083B, IR 67684 B, IR 58025B, Ajaya R, IR
		59624-34-2-2 R , IR 59682-132-1-1-2R , IR 62171-122-3-2-3-
		2R, IR 63870-3-2-3-3R, IR 68926-61-1R, IR 44675 R, IR
		65515-19R, IR 60819-34-2-1R, IR 60236-222-3-3-1-2R, IR
		63879-195-2-2-3-2R , IR 36

Table 4. Average intra and inter cluster distances (D^2) of 50 parental lines along with 2 check varieties (during aman season at 14 DAI)

Cluster	Ι	II	III	IV	V	
Ι	0.385					
II	4.012	0.237				
III	17.500	13.491	0.819			
IV	6.306	2.302	11.194	0.324		
V	11.794	7.782	5.717	5.494	0.409	

Bold figures denote the intra cluster distance

Table 5. Cluster mean for 2 characters in 50 parental lines along with 2 check varieties (during aman season at 14 DAI)

Characters	Clusters				
	Ι	II	III	IV	V
Lesion length (cm)	18.9	14.34	3.43	12.97	7.21
Relative lesion length (%)	90.31	72.43	12.81	62.41	37.84

Table 6. Characters contribution towards divergence (during aman season at 14 DAI)

Characters	Vector I	Vector II
Mean Lesion Length	-0.0564	0.2999
Relative Lesion Length	0.2365	-0.0590

Table 7. Average intra and inter cluster distances (D^2) of 50 parental lines along with 2 check varieties (during boro season at 14 DAI)

Cluster	Ι	II	III	IV	V	
Ι	0.528					
II	3.550	0.214				
III	1.725	1.827	0.215			
IV	16.786	13.269	15.071	0.229		
V	7.735	4.186	6.010	9.123	0.340	

Bold figures denote the intra cluster distance

Table 8. Cluster mean for 2 characters in 50 parental lines along with 2 check varieties (during boro season at 14 DAI)

Characters		Clusters			
	Ι	II	III	IV	V
Lesion length (cm)	0.527	2.160	1.354	9.866	4.219
Relative lesion length	1.686	7.618	4.617	32.056	14.810
(%)					

Table 9. Characters contribution towards divergence (during boro season at 14 DAI)

Characters	Vector I	Vector II	
Mean Lesion Length	-1.321	-2.221	
Relative Lesion Length	0.959	0.674	



Fig. 1. Scatter diagram of 50 parental lines along with 2 check varieties based on their principal component scores superimposed with clustering (during aman season at 14 DAI).



Fig. 2. Scatter diagram of 50 parental lines along with 2 check varieties based on their principal component scores superimposed with clustering (during boro season at 14 DAI).

References

- Ali, A., Khan, M. H., Bano, R., Rashid, H., Raja, N. I. and Chaudhry, Z. (2009). Screening of Pakistani rice (*Oryzae sativa*) cultivars against *Xanthomonas oryza* pv. *oryzae*. Pakistan Journal of Botany 41:2595-2604.
- Bedi, K. S. and Gill, H. S. (1960). Losses caused by the brown leaf spot disease of rice in Punjab. Indian Phytopathology 13:161-164.
- Das, B. C., Bora, L. C. and Bhagwati, K. N. (1994). Interaction of virulent and avirulent isolates of *Xanthomonas oryzae* pv. *oryzae* with rice chloroplast, variation in lesion development and electrolyte leakage. Indian Journal of Mycology and Plant Pathology 24:29-32.
- Ezuka, A. and Kaku, H. (2000). A historical review of bacterial blight of rice. Department of Genetic Resources II and I. Bulletin of the National Institute of Agrobiological Resources 15:1-207.
- Fang, Z. D., Xu, Z. G., Guo, C. J., Yinm, S. Z., Wu, S. Z., Xu, Z. M. and Jhang, Q. (1990). Studies on pathotypes of *Xanthomonas campestris* pv. *oryzae* in China. Acta Phytopathologica Sinica 20:81-87.
- Karki, P. B. (1991). Rice response to bacterial leaf blight. Journal of the Institute of Agriculture and Animal Science 12:115-119.
- Kauffman, H. E., A. P. K. Reddy, S. P. Y. Hsieh, S. D. and Merca, S. D. (1973). An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. Plant Disease Reporter 57:537-541.
- Khan, M. A. I, Mansur, M. A., Ali, M. A., and Mia, M. A. T. (2009). Pathogenic diversity of *Xanthomonas oryzae* pv. *oryzae* in Bangldesh. Bangladesh Journal of Plant Pathology 25:1-6.
- Khush, G. S. and Ogawa, T. (1989). Major gene for resistance to bacterial blight in rice. In: bacterial blight of rice. The International Rice Research Institute, P.O. Box 933, Manila, Philippines. pp. 177-192.
- Mew, T. W. (1987) Current status and future prospects of research on bacterial blight of rice. Annual Review of Phytopathology 25:359-382.
- Mew, W. T. (1993). Study on rice bacterial blight syndrome. International research institute, P.O. Box 933, Manila, Philippines
- Mohanty, A. K., Sethi, P. N. and Sahu, A. K. (1996). Screening new semi deep water rice cultivars for reaction to bacterial leaf blight disease. Journal of Mycopathological Research 34:93-97.
- Mokate, A. S., Mehetre, S. S., Bendale, V. W. and Birari, S. P. (1998). Genetic divergence in rice. Advances in plant sciences 11:189-192.
- Mondal, A. H. and Hossain, M. A. (1997). Screening for Resistance against Rice Bacterial blight in Bangladesh. Bangladesh Journal of Botany 26:25-29.
- Ogawa, T., Yamamoto, T., Khush, G. S. and Mew, T. M. (1991). Breeding of near isogenic lines of rice with single genes for resistance to bacterial blight pathogen (*Xanthomonas campestris* pv. *oryzae*). Japanese Journal of Breeding 41:523-529.
- Ou, S. H. (1985). Rice Diseases. 2nd edition. CMI, Kew, Surrey, U.K. 380 pp.
- Salim, M., Akram, M., Akhtar, M. E. and Ashraf, M. (2003). A Production Handbook, Balaced Fertilization for Maximizing economic Crop yields. Pakistan Research Council, Islamabad. 1-8 pp.

- Shah, S. M. A., Rahman, H., Abassi, F. M., Akhtar, M. A., Rafi, A. and Khan, I. A. (2009). Resistance characterization of wild relatives of rice in response to bacterial blight. Pakistan Journal of Botany 41:917-925.
- Shahjehan, A. K. M., Ahmad, H. U., Mia, M. A. T., Sharma, M. A. and Nahar, N. S. (1991). Out break of leaf blight in rice crop in Bangladesh. Iran. 21 pp.
- Singh, G. P., Srivastava, M. K., Singh, R. M. and Singh, R. V. (1977). Variation in quantitative and qualitative losses caused by bacterial blight rice varieties. Indian Phytopathology 30:180-185.
- Tabei, H. (1997). Anatomical studies of rice plant affected with bacterial leaf blight, *Xanthomonas oryzae* pv. *oryzae* (Uyeda et Ishiyama) Dowson. Bullet of the. Kyushu National Agricultural Experiment Station 19:193-257.
- Virmani, S. S. (1996). Hybrid Rice. Advances in Agronomy. 379 pp.
- Wei, W. X., Zhang, H., Lu, F. U. and Wei, S. L. (1994). Principal component analysis and genetic distance estimation and their application in sesame breeding programme. Acta Agriculturse Boreali Sinica 9:29-33.
- Zhang, Q., Wang, C. L., Shi, A. N., Bai, J. F., Ling, S. C., Li, D. Y., Chen, C. B. and Pang, H. H. (1994). Evaluation of resistance to bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) in wild rice species. Scientia Agricultura Sinica 27:1-9.
- Zhao, X. H., Shen, X. P. and Zhu, Y. (1994). Reaction of foreign rice varieties to pathotypes of bacterial leaf blight and their utilization. Entomological Knowledge 31:2-3.

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